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Effects of Thyroidectomy, Hypophysectomy, Temperature and Humidity on the Occurrence of Nocturnal Locomotor Activity in the Toad, *Bufo japonicus*, during the Breeding Season

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ABSTRACT—Toads collected in October 1987 were thyroidectomized or hypophysectomized, and then released into an outdoor pen. Observations were made once a day between 2030 and 2130 hr for 19 days from February 27 to March 16, 1988. Toads found completely exposed above ground at the time of the observation were regarded as active individuals. In males thyroidectomy significantly reduced both the mean number of active individuals each day and the mean number of active days of each individual. Hypophysectomy significantly reduced both of these parameters in males. In females, the effects of the operations were not clear, since the number of active individuals was extremely small in all groups. Significant positive correlations ($r=0.58$–0.76) were observed between the number of active individuals and temperature in both sexes, and between the number of active individuals and the humidity of the air in males. When the temperature and humidity were combined as an independent variable, a highly significant multiple correlation ($r>0.8$) in both sexes was observed. The present results suggest that the pituitary gland plays some role in the migration of the toad toward the pond but thyroid hormone suppresses the migratory activity, and also that the combination of temperature and humidity is the external factor which initiates the migration.

INTRODUCTION

In early spring, adult toads, *Bufo japonicus*, come out of hibernation and begin migration to a particular pond for breeding. This migratory activity occurs only on warm and humid nights and hence, not every day. Temperature and humidity have been proposed as the atmospheric factors which initiate the migration [1, 2]. A possible candidate for the endocrine factor initiating the migration for breeding in amphibians was prolactin, since a number of investigators have shown that prolactin is the water drive factor in newts and salamanders [3–8]. However, one of the present authors and his associates have found that the plasma prolactin level of toads just before or just beginning migration toward a breeding pond was low, and hence, prolactin can not be the migration inducing factor in the toad [9, 10]. Tasaki *et al.* [11], observing the elevation of plasma thyroxine and triiodothyronine levels during the breeding season in *Bufo japonicus*, suggested the possibility that thyroid hormone is the migration inducing factor in the toad. However, they recently found that thyroxine administration to normal and thyroidectomized male toads suppressed their locomotor activity when measured in a small chamber, and thyroidectomy of male toads increased the activity [12]. These results, though under experimental conditions, suggest that thyroid hormone also can not be the migration inducing factor of the toad.

The purpose of the present study, is to confirm our previous results under conditions which approximate the natural environment, and also to determine the role of the pituitary gland in the migration. The effects of thyroidectomy and hypophysectomy on the occurrence of nocturnal locomotor activity were studied in toads kept in an

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outdoor pen during the breeding season.

MATERIALS AND METHODS

Material

Adult male and female toads (*Bufo japonicus*) were used. They were captured in the suburbs of Tokyo in October 1987. The mean body weight was 145.2 g with a standard deviation of 42.0 g.

Operations

After capture, male and female toads were put in separate plastic boxes ($55\times40\times43\ cm^3$) with loose fitting tops. Wet pieces of plastic sponge were put in the boxes to maintain humidity. No feeding took place, since toads abstain from food during the hibernation and breeding periods. Toads were kept in the boxes outdoors for about 2 weeks before the operations. Eight males and eight females were thyroidectomized. Sham-operations were performed on the same number of animals of each sex. Hypophysectomy by the oral approach and corresponding sham-operations were also performed with the same number of male and female toads. MS-222 was used for anesthetization of toads. Each animal was individually marked with a small, numbered plastic sheet which was adhered to its back, and a numbered plastic band which was tied around a forelimb.

Observation of toads

Fourteen to eighteen days after the thyroidectomy and related sham operation or 8 to 11 days

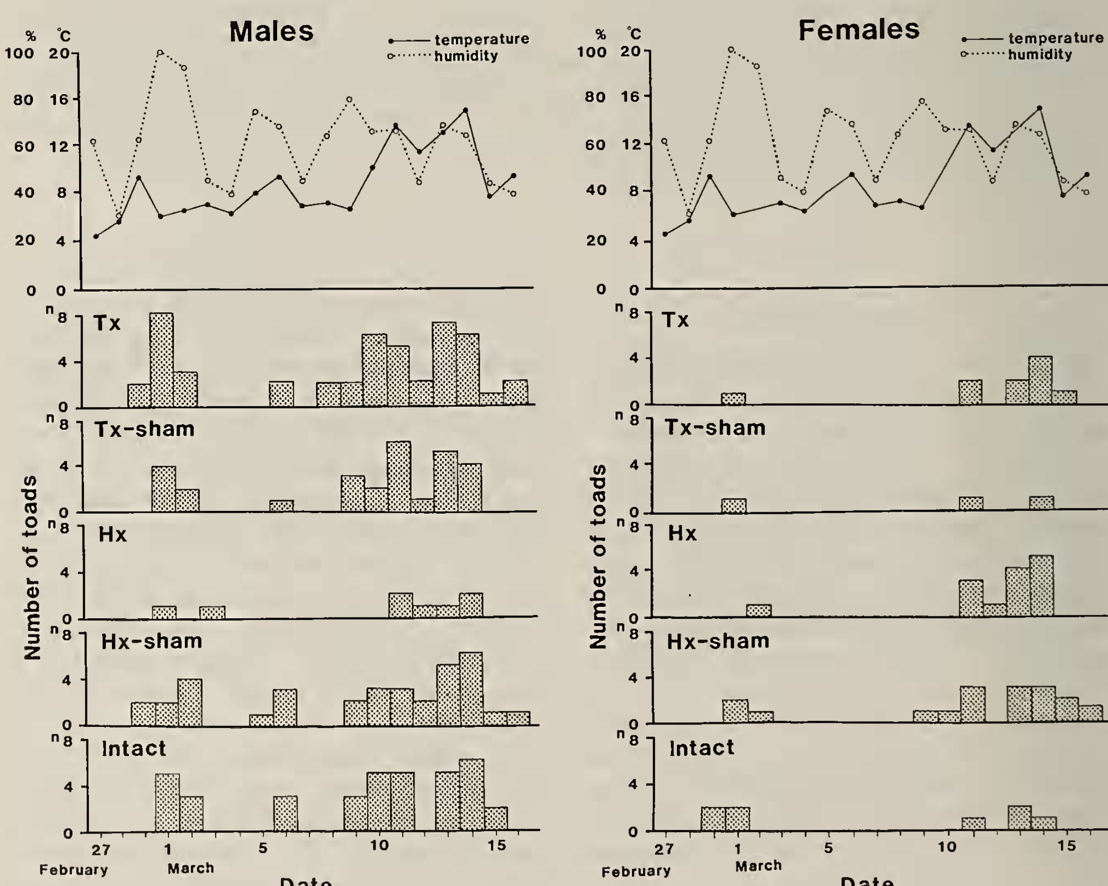

Fig. 1. Atmospheric factors and the number of male (left) and female (right) toads which appeared above ground on each experimental day in each group.

after the hypophysectomy and related sham operation, toads were released into an outdoor pen ($2 \times 26\,m^2$). As soon as they were released, they buried themselves under the ground. Observations were made in the pen once a day between 2030 and 2130 for 19 days from February 27 to March 16, 1988. When eggs were laid by a female in a pool ($70 \times 42 \times 20\,cm^3$) in the pen on March 16, observations were terminated. Toads which were found completely exposed above ground were regarded as active individuals, and their identification numbers were recorded. The temperature and relative humidity 10 cm from the ground surface in a corner of the pen were recorded every day at the time of the observation.

Statistical methods

The significant over-all difference in the number of active toads among the groups was determined by Friedman's test. The significant difference in the number of active toads between two groups was determined by the signed-rank test. The randomization test was used to compare the two groups as to the number of days on which toads showed activity. For these tests, personal computer programs [13] were employed.

RESULTS

Effects of thyroidectomy and hypophysectomy on the occurrence of activity

The daily number of male and female toads of each group which appeared above ground for locomotor activity is shown in Figure 1. The temperature and relative humidity are indicated in the figure. Using the same data, the number of days on which each toad showed activity was calculated, and its distribution is shown in Figure 2.

Males: In males, the locomotor activity was observed relatively frequently, i.e., 15 out of 19 days. However, the number of active toads fluctuated widely over the course of the days and also among the groups (Fig. 1). The over-all difference in the number of active toads each day among the five groups was tested by using Friedman's test. The difference was highly significant ($p<0.01$).

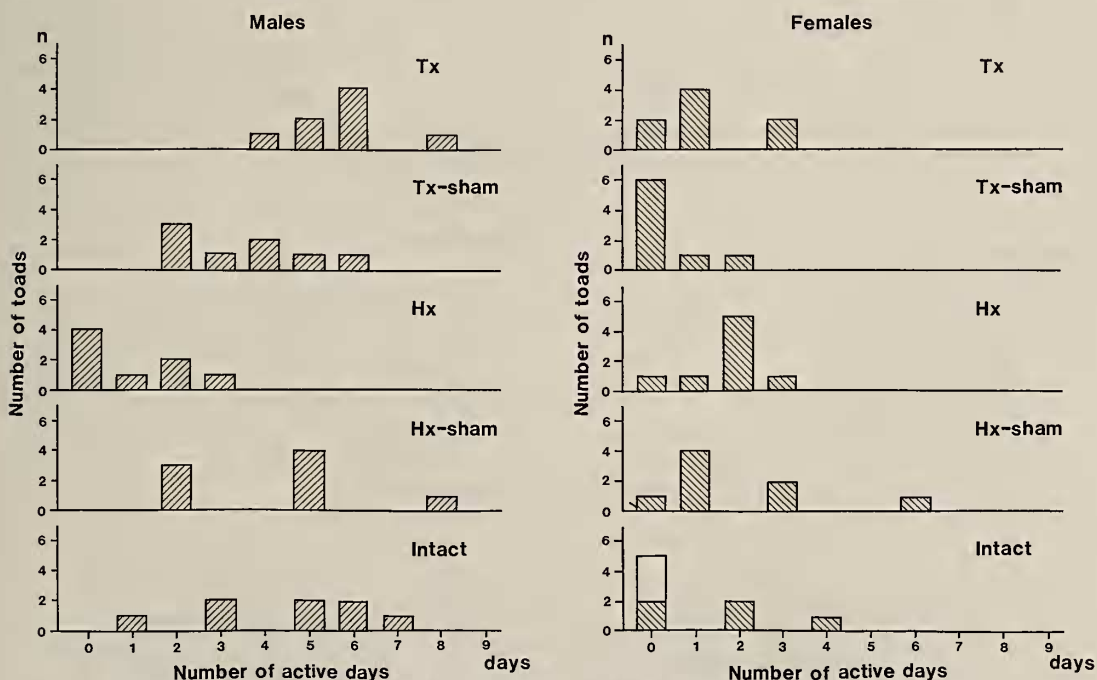

FIG. 2. The distribution of the number of days in which male (left) and female (right) toads showed activity. The open column indicates the number of toads which were not recovered after the observation period.

Then, the two-sided signed-rank test was used for paired comparisons between two selected groups. The differences in the number of active males between the thyroidectomized and its sham-operation control groups and also between the hypophysectomized and its sham-operation control groups were highly significant ($p<0.01$). The differences between each of the sham-operation groups and the intact group were not significant ($p>0.05$).

The distribution of the number of days on which the toad showed activity is indicated in Figure 2. The difference in the average number of active days between two groups was tested by the two-sided randomization test. Thyroidectomized males showed activity more frequently than corresponding sham-operation males ($p<0.008$), but hypophysectomized males showed it less frequently than the corresponding sham-operation males ($p<0.002$). The frequency did not differ significantly ($p>0.05$) between each of the sham-operation groups and the intact group.

Females: Compared to males, a fewer number of females showed locomotor activity (cf. Fig. 1).

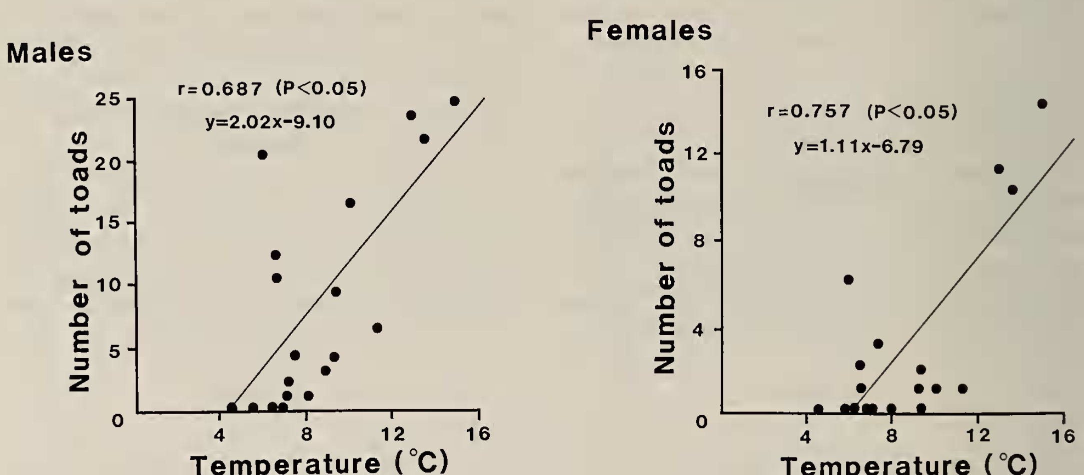

FIG. 3. The single correlation between temperature and the number of active male (left) and female (right) toads. There was a significant ($p<0.05$) positive correlation in both males and females.

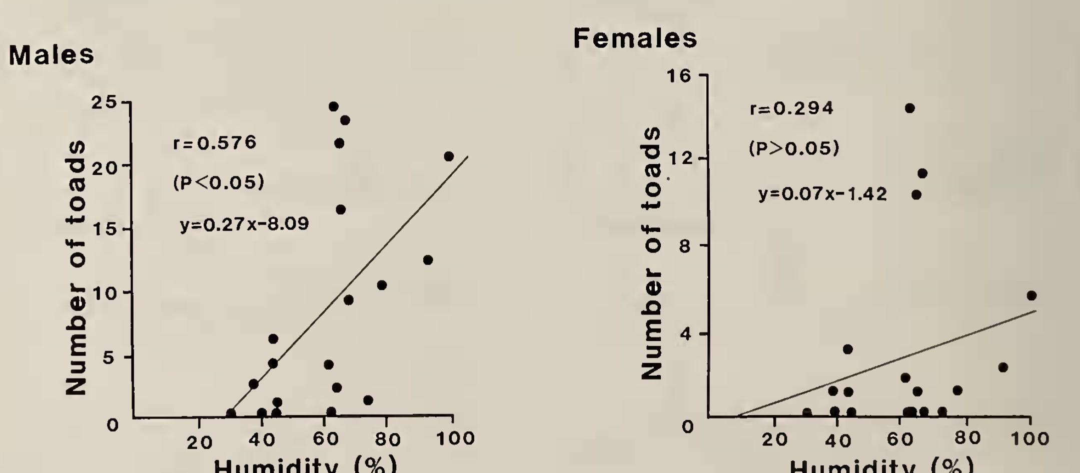

FIG. 4. The single correlation between humidity and the number of active male (left) and female (right) toads. Only in males did the humidity show a significant ($p<0.05$) positive correlation.

The mean number of active days for females was also less than that for males (cf. Fig. 2). Due to the small number of active individuals, no significant difference was obtained or no statistical test was valid for the comparison of the number of active toads or the number of active days among the groups.

Correlations between atmospheric parameters and toad activity

The effect of temperature or humidity on the number of active toads was studied by simple

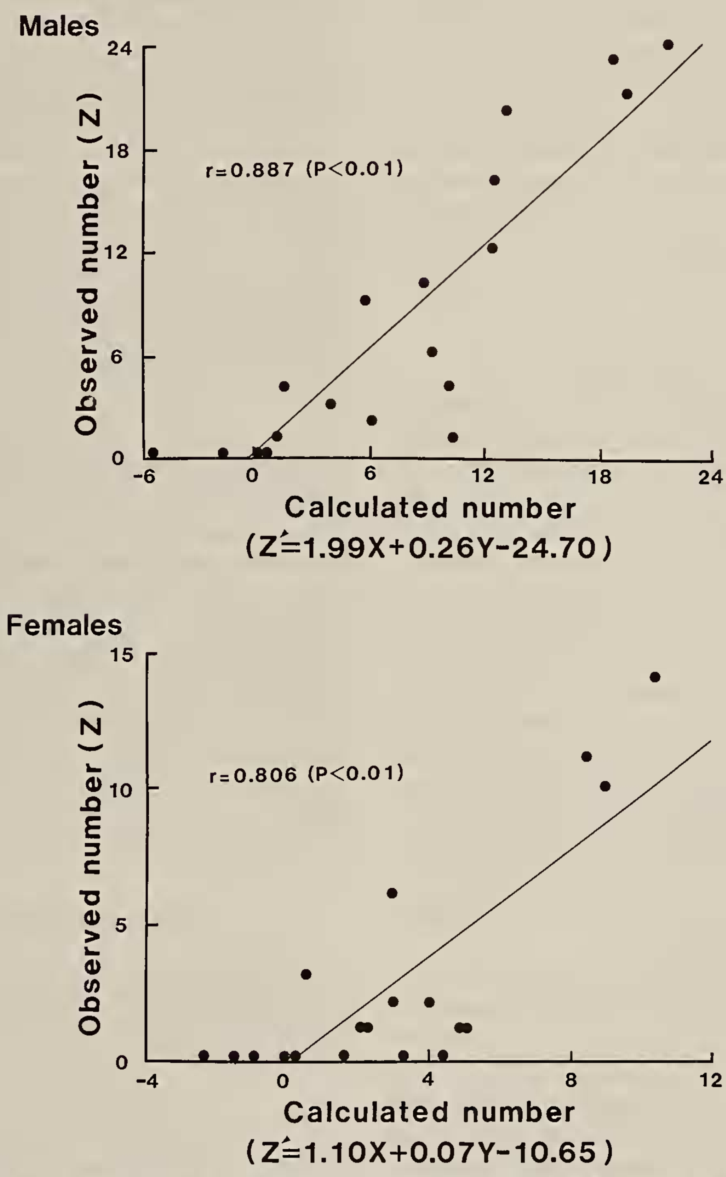

FIG. 5. The multiple correlation among temperature, humidity and the number of active male and female toads. A highly significant multiple correlation was observed between the temperature plus humidity and the number of active toads. On the horizontal axis(Z'), the expected numbers of active toads, which were calcuated by using the regression formula with observed temperature(X) and humidity(Y) values, are plotted. On the vertical axis(Z), the observed numbers of active toads are plotted.

correlation analysis. The combined effect of both temperature and humidity was also studied by multiple correlation analysis. Females and males were separately analyzed.

As depicted in Figure 3, temperature showed a significant ($p<0.05$) positive correlation with the number of active toads in both males and females. Humidity showed a significant positive correlation in males only (Fig. 4). In females, the correlation coefficient was positive, but it was too small to be statistically significant ($p>0.05$, Fig. 4).

When both temperature and humidity were used as independent variables, a highly significant multiple correlation ($p<0.01$) with the number of active toads was observed in males as well al in females (Fig. 5).

DISCUSSION

A number of investigations have been published on the role of thyroid hormones in the migration of vertebrates: in fish [14–15], amphibians [5, 7, 16, 17] and birds [18]. Our observations on the annual cycle of plasma thyroxine and triiodothyronine levels in the toad, *Bufo japonicus,* seemed to support the idea that thyroid hormone plays a role in toad migration. On the contrary, we recently observed that the treatment of male *Bufo japonicus* with thyroxine decreased the distance of locomotion of a toad kept in a small experimental chamber [12]. This effect of thyroxine was observed in both normal and thyroidectomized males, and thyroidectomy increased the distance of locomotion. However, we were cautious in concluding that the sedative effect of thyroxine was physiological, since the effect was observed under artificial conditions.

Our former results of the thyroidectomy experiment [12] were confirmed under the quasi-natural conditions in the present study. The thyroidectomy increased both the number of toads which showed activity and the number of days on which the toads showed activity. Accordingly, we conclude that thyroxine decreased both parameters representing the occurrence of locomotor or migratory activity during the breeding season even under the quasi-natural conditions. However, it is not known whether the effect of thyroxine is direct or indirect. Furthermore, the physiological meaning of this effect is still obscure, although we postulated that it might be related to post-breeding inactiveness [12].

Hypophysectomy clearly suppressed the activity of male toads. This suggests that some hormone(s) of the pituitary gland activates(s) migratory activity in male toads. However, it is difficult to conclude whether this suppressive effect of hypophysectomy is due to the ablation of a specific hormone controlling the migration or the ablation of hormone(s) regulating the general metabolism.

Another problem was the inactiveness of the female toads, which caused difficulty in observing the effect of the operations in females. Only a few females showed activity in the present study as well as in the previous study in the chamber [12]. This may be related to the fact that not all female toads participate in breeding activity every spring [19–21].

It is revealed in the present study that the combination of temperature and humidity is the external factor which initiates locomotor activity in early spring. The high humidity may be advantageous for gas exchange through the skin of the toad and the relatively high temperature, for energy metabolism. A high rate of this external and internal respiration is necessary for muscular activity during the migration of toads in early spring. We can now predict the occurrence of migration in toads from the temperature and humidity by using the regression formula obtained in the present study.

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REFERENCES

1 Hisai, N. and Sugawara, T. (1978) Ecological studies of *Bufo bufo japonicus* Schlegel (V) The relation between appearances and the climatic conditions at breeding season. Miscellaneous Reports of the National Park for Nature Study, **8**: 135–149. (In Japanese)

2 Okuno, R. (1985) Studies on the natural history of the Japanese toad, *Bufo japonicus japonicus.* VIII. Climatic factors influencing the breeding activity. Jap. J. Ecol., **35**: 527–535. (In Japanese)

3 Reinke, E. E. and Chadwick, C. S. (1940) The origin of the water drive in *Triturus viridescens.* J. Exp. Zool., **83**: 223–233.

4 Chadwick, C. S. (1944) Further observations on the water drive in *Triturus viridescens.* J. Exp. Zool., **86**: 175–187.

5 Crim, J. W. (1975) Prolactin-induced modification of visual pigments in the eastern red-spotted newt, *Notophthalmus viridescens.* Gen. Comp. Endocrinol., **26**: 233–242.

6 Duvall, D. and Norris, D. O. (1977) Prolactin and substrate stimulation of locomotor activity in adult tiger salamanders (*Ambystoma tigrinum*). J. Exp. Zool., **200**: 103–106.

7 Duvall, D. and Norris, D. O. (1980) Stimulation of terrestrial-substrate preferences and locomotor activity in newly transformed tiger salamanders (*Ambystoma tigrinum*)by exogenous or endogenous thyroxine. Anim. Behav., **28**: 116–123.

8 Moriya, T. (1982) Prolactin induces increase in the specific gravity of salamander, *Hynobius retardatus,* that raises adaptability to water. J. Exp. Zool., **223**: 83–88.

9 Yoneyama, H., Ishii, S., Yamamoto, K. and Kikuyama, S. (1984) Plasma prolactin levels of *Bufo japonicus* before, during and after breeding in the pond. Zool. Sci., **1**: 969.

10 Ishii, S., Yoneyama, H., Inoue, M., Yamamoto, K. and Kikuyama, S. (1989) Changes in plasma and pituitary levels of prolactin in the toad, *Bufo japonicus,* throughout the year with special reference to the breeding migration. Gen. Comp. Endocrinol., **74**: 365–372.

11 Tasaki, Y., Inoue, M. and Ishii, S. (1986) Annual cycle of Plasma thyroid hormone levels in the toad, *Bufo japonicus.* Gen. Comp. Endocrinol., **62**: 404–410.

12 Tasaki, Y. and Ishii, S. (1989) Effects of thyroxine on locomotor activity and carbon dioxide release in the toad. *Bufo japonicus.* Zool. Sci. (In press)

13 Ishii, S. (1983) “Programs of statistical methods for biologists by N88-BASIC”, Baifukan, Tokyo.

14 Woodhead, A. D. (1975) Endocrine physiology of fish migration. Oceanogr. Mar. Biol. Annu. Rev., **13**: 287–382.

15 Dickhoff, W. W., Folmar, L. C. and Gorbman, A. (1978) Changes in plasma thyroxine during smoltification of coho salmon, *Oncorhynchus kisutch.* Gen. Comp. Endocrinol., **36**: 229–232.

16 Grant, W. C., Jr. and Cooper, G. IV (1965) Behavioral and integumentary changes associated with induced metamorphosis in *Diemictylus.* Biol. Bull., **129**: 510–522.

17 Dent, J. N. (1985) Hormonal interaction in the regulation of migratory movements in urodele amphibians. In “The Endocrine System and the Environment”. Ed. by B. K. Follet, S. Ishii and A. Chandola, Japan Sci. Soc. Press, Tokyo/Springer-Verlag, Berlin, pp. 79–84.

18 Berthold, P. (1985) Migration: Control and metabolic physiology. In “Avian Biology”, Ed. by D. S. Farner and J. R. King, vol. 5, Academic Press, New York, pp. 77–128.

19 Hisai, N. (1981) Ecological studies of *Bufo bufo formosus* Boulenger (VI) Differences of postmetamorphic growth rate and the sexual maturity between sexes in the natural population. Miscellaneous Reports of the National Park for Nature Study, **12**: 103–113. (In Japanese)

20 Okuno, R. (1985) Studies on the natural history of the Japanese toad, *Bufo japonicus japonicus.* V. Post-metamorphic survival and longevity. Jap. J. Ecol., **35**: 93–101. (In Japanese)

21 Okuno, R. (1986) Studies on the natural history of the Japanese toad, *Bufo japonicus japonicus.* IX. Male behaviors during breeding season. Jap. J. Ecol., **35**: 621–630. (In Japanese)